2008). Cyclooxygenase-Inhibiting Nitric Oxide Donators (CINODs) are a new class of anti-arthritic drugs, which inhibit in a balanced way both COX-1 and COX-2 while releasing nitric oxide, an important modulator of vascular tone. We present here the effects of HCT 1026, a flurbiprofen-based CINOD, on chondrocytes, focusing on their catabolic and anabolic activities, as well as activation of inflammatory pathways.
Methods: Isolated primary cultures of bovine and human articular chondrocytes (BAC and HAC) were preincubated with 10 μM HCT 1026 or flurbiprofen and stimulated with Il-1β, in hypoxic or normoxic conditions. Levels of mRNA for extracellular matrix proteins (aggrecan and collagen type II) and matrix metalloproteases (MMPs), as well as TGFβ receptor, were determined by quantitative RT-PCR. NO release was assessed on cell supernatants through the Griess reaction, and activities of NF-κB and AP-1 were determined by electrophoretic mobility shift assay. NO donation from HCT 1026 was evaluated by testing its vasorelaxing activity in norepinephrine-precontracted rabbit aortic rings, in presence or absence of 10 μM ODQ (a specific inhibitor of NO-dependent cGMP formation).
Results: In hypoxic human articular chondrocytes stimulated with Il-1β, both HCT 1026 and flurbiprofen decreased expression of MMP-1 and -3 and TGF-β receptor by 20 to 40%. However, only HCT 1026 inhibited Il-1β-dependent NO overproduction while did not affect basal aggrecan or type II collagen mRNAs. Moreover, in BAC cultured in normoxic conditions, HCT 1026 inhibited NF-κB and AP-1 activation, whereas flurbirprofen affected only AP-1 activity. HCT 1026, but not flurbiprofen, induced aorta vasorelaxation, with an EC_{50} of 5.9±1.2 μM. ODQ pretreatment abolished this effect, confirming that vascular relaxation by HCT 1026 was based on its NO donating properties.
Conclusions: The CINOD HCT 1026 modulates chondrocytes catabolic and anabolic metabolism, causing reduction of inflammatory markers such as NO biosynthesis and activation of NF-κB. Moreover, HCT 1026 is able to donate biologically relevant nitric oxide, which can modulate vascular tone. If confirmed in *in vivo* studies, our data may provide evidence that the ability of CINODs to donate NO have a potential beneficial impact on joint cartilage in OA patients.

542 EFFECTIVENESS OF MELOXICAM FOR TREATMENT OF PAIN SYNDROME UNDER KNEE OSTEOARTHRITIS, LOW BACK PAIN AND NECK PAIN IN ELDERLY PEOPLE

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Purpose: The research was aimed at evaluating of the effectiveness of meloxicam (revmoxicam) for treatment of pain syndrome under knee osteoarthritis, low back pain and neck pain in elderly people.
Methods: The patients were devided into three groups. The first group included 11 patients (aged 61.1±5.54 years) with neck pain, the second group included 10 patients (aged 59.4±4.65 years) with low back pain and the third group included 10 patients (aged 57.9±7.5 years) with knee osteoarthritis. All patients took meloxicam 15 mg once per day during 5 days. The following methods of study were used: Mc-Gill questionnaire, VAS, WOMAC, determination of life quality by Roland-Morris questionnaire, Neck disability index, EuroQol 5D scale.

Table 1. Dynamics of pain syndrome intensive after meloxicam treatment in patient of different groups.

Index	Group	Before treatment	After 5 days of treatment	P	Two weeks without treatment	P
Index of pain, cm	I	444±0.71	4.11±0.78	0.35	3.22±3.00	0.01
	II	5.13±0.59	4.13±0.79	0.03	3.63±0.52	0.003
	III	5.98±2.22	3.56±1.74	0.001	3.66±1.86	0.043
Descriptors, ball	I	9.56±2.23	7.56±2.40	0.09	7.56±1.71	0.12
	II	11.0±2.29	8.63±2.79	0.016	9.63±3.65	0.49
	III	10.60±4.70	7.40±4.80	0.008	8.70±6.00	0.043
Ranks, ball	I	18.78±5.17	13.22±5.28	0.07	12.00±3.52	0.03
	II	23.50±6.71	16.88±6.61	0.006	18.88±8.06	0.36
	III	23.30±16.10	16.50±11.70	0.01	18.50±15.0	0.57

Results: After 5 days of treatment patients, who was taking meloxicam, noticed a significant decrease of pain syndrome and it became low after two weeks without treatment [Table].
Significant decrease of all parameters of pain syndrome in second and third groups was observed after 5 days. Intensity of pain syndrome was also certainly decreased after two weeks without treatment in all groups by some parameters. The difference of effectiveness of meloxicam in patient with different diseases was observed by VAS: decrease of pain syndrome in the first group was – 7%, in the second groups – 17%, and in the third group – 39%, F = 2.76, p = 0.08. During the period of research no patients who undergone treatment had registered any side effects. There was no significant difference between groups dealing with the improvement of patient's everyday activity.
Conclusions: It can be concluded that the meloxicam is effective and safe in the treatment of low back pain syndrome, neck pain syndrome and pain syndrome under knee osteoarthritis in elderly people. However, the best effect of meloxicam was observed in patients with pain syndrome under knee osteoarthritis that can be explained by the inflammatory component of osteoarthritis pathogenesis.

543 EFFECTIVENESS OF TWO REGIMES OF GLUCOSAMINE AND CHONDROITIN FOR TREATMENT OF PAIN SYNDROME IN PATIENT WITH KNEE OSTEOARTHRITIS

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Purpose: The research was aimed at evaluating the effectiveness of two regimes (continuous and interrupted) of Theraflex (500 mg glucosamine hydrochloride, 400 mg chondroitin sulphate) in patients with knee osteoarthritis. Outcomes evaluated were pain, measures of performance (function, activity of daily living, disability), employment status, range of motion, and patient satisfaction/patient global perceived effects.
Methods: The first group included 50 patients (aged 64.5±1.1 years) with knee osteoarthritis (II stage, Kellgren-Lawrence's classification), who took the drug in continuous regime during 9 months. The second group included 50 patients with the same diagnosis (aged 64.6±1.0 years), who took Theraflex twice during 3 months with 3 months interruption. We examined the patients before the treatment and after 1, 3, 6, 9 and 12 months. Methods of study: Mc-Gill questionnaire, visual-analogical scale (VAS), Lequen's index, WOMAC, EuroQol-5D, 15-m. test, 6-min. test.
Results: After three months of Theraflex's treatment it was observed a reliable decrease of pain syndrome in both groups by WOMAC, decrease of constraint in movements, improvement of index of everyday activity, VAS, 15-m.test. Examination of patients during 6, 9 and 12 months show the effectiveness of both regimes of the therapy. Intensity of pain syndrome and functional activity didn't differ between the groups.
Conclusions: During 1-year period two regimes of Theraflex it was established effective decrease of intensity of the pain syndrome and improvement of everyday activity in patients with knee osteoarthritis. The analgesic effect after taking Theraflex becomes noticeable after three months and quality of life significantly improved in patients of both groups.

544 THE FLEXX TRIAL OF OSTEOARTHRITIS OF THE KNEE: INTRA-ARTICULAR HYALURONAN (EUFLEXXA™)

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Purpose: The FLEXX trial studied the 6 month efficacy and safety of a non-avian form of IA hyaluronan for OA of the knee.
Methods: This was a randomized, saline-controlled, double-blind study of 586 patients with primary OA of the knee were evaluated prior to and following 3 weekly IA injections of hyaluronan or buffered saline. The primary efficacy measurement was pain recorded for the signal knee after a 50-foot walk. Secondary variables included the 3 subscales of the WOMAC, OARSI responder criteria, patient global assessment, use of rescue medication, quality of life (SF-36), and safety.
Results: The ITT population included Hyaluronan n = 291 and Saline n = 295. There were 12% dropouts (Hyaluronan n = 34; Saline n = 34). Hyaluronan demographics: Age 62.5±10.6 (SD), women 63%, BMI 32±7, Kellgren-Lawrence grade 2−41%, grade 3−59%, initial pain 56±22 with minimal differences from the saline group. After 26 weeks, there was a significant improvement in pain recorded after a 50-foot walk (ANCOVA; p = 0.028). Secondary variables were generally supportive of the primary measurement. Serious adverse events occurred in Hyaluronan (n = 10) and Saline (n = 9) groups. Arthralgia occurred in Hyaluronan (n = 27) and Saline (n = 35) groups. Local injection site reactions occurred in Hyaluronan (n = 2) and Saline (n = 1) groups.
Conclusions: IA hyaluronan was significantly superior to saline for OA of the knee over a 6-month period despite a large beneficial effect of IA saline.

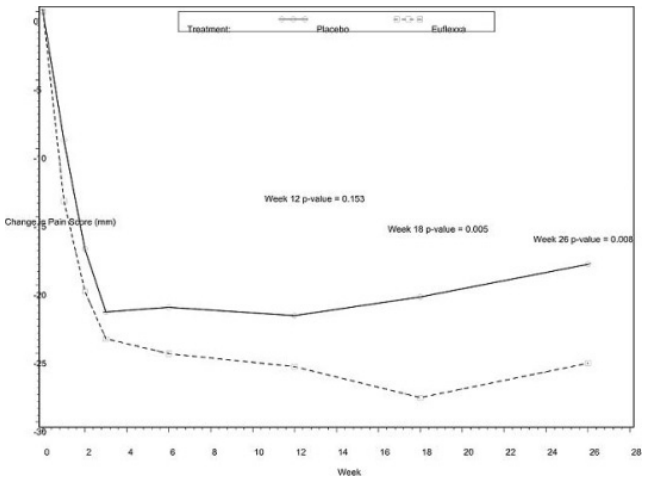

Figure 1. Change in pain scores on the 50-foot Walk Test from baseline to week 26 ITT population.

545 SODIUM PENTOSAN POLYSULFATE BROUGHT ABOUT CARTILAGE IMPROVEMENT IN KNEE OSTEOARTHRITIS

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Purpose: Within the last few decades the concept of disease modifying drugs (DMOAD) have been explored as alternative therapeutic treatment modalities for osteoarthritis (OA). From the results of previous in vitro and animal model studies, we have proposed that the spectrum of pharmacological activities exhibited by Sodium Pentosan Polysulfate (NaPPS) would qualify it as a DMOAD. However human clinical evidence to support this proposition was a few. To assess the efficacy, safety, and patient satisfaction with a series of subcutaneous injections of NaPPS in the patients who reported knee joint OA-associated pain, loss of ROM, and disability that interfered with daily life and work

Methods: NaPPS is a semi-synthetic drug manufactured from beechwood hemicellulose by sulfate esterification of the xylopyranose hydroxyl groups. It has an average mean molecular weight of 5700 Da. NaPPS in this study was manufactured and supplied in injectable vials (100 mg/ml) by bene-Arzneimittel GmbH, Munich, Germany.

The open trial was undertaken with 20 patients with knee OA. Patients were clinically assessed at entry and 1, 2, 3, 4, 8, 12, 16, 24 and 52 weeks with physical examination and VAS for stiffness, pain at rest or walking, ROM exercises, walking up and down stairs. A modified WOMAC OA index was used for lifestyle parameters. Degradation of type II Collagen (C2C) in Blood was measured with an ELISA kit, in accordance with the manufacturer's instructions.

Before the treatment, 2 weeks of NSAIDs washout period was made, if necessary. Treatment consisted of 6 weekly subcutaneous injections (sc) of NaPPS (2 mg/kg). The protocol and written informed consent for studies involving human subjects were approved by the review board in our university. The statistical significance, compared with the value at entry, was determined by one way ANOVA and Dunnett's method.

Results: The attribution of patients was as following: The average year was 63 years old, All cases were female, were included in grade 1 to 2 according the classification of Kellgren and Lawrence. The average of WOMAC score at the first visit was 37.0. All cases could be followed for one year.

The dose of this PPS effected the blood coagulation test, but the value in the study was within safety area. A tiny abnormal finding is noted in serum chemistry: ie: serum triglyceride 1 hr after injection., but it was reduced quickly in followed up period.

The hydroarthroses were reduced quickly in all cases. ROM of the knee joint was improved. The clinical assessments, i.e. knee flexion, pain at walking, pain just after climb up and down stairs, pain just after ROM exercise were improved significantly. The concentration of C2C in Blood were decreased significantly. The clinical effect was continued for almost one year.

The improvement of X ray findings could not detected.

In the previous vitro study, PPS reduced cartilage degradation by directly and indirectly affecting these inflammatory mediators such as MMP-3. PPS increased both the synthesis and the molecular weight of hyaluronan. PPS had anti-inflamatory function. As the results of C2C in Blood, quickly reducion of hydroarthroses and improvement of the knee joint R.O.M., our result was compatible to the above vitro study

Conclusions: The availability, safety of NaPPS for knee osteo-arthlitis patients and satisfaction level of patients was studied.

546 DUAL INHIBITION OF Il-1-INDUCED NF-κB ACTIVATION AND iNOS ENZYME ACTIVITY, IN HUMAN CHONDROCYTES, BY NATURAL AND COMMERCIAL α-PINENE

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Purpose: Our previous studies showed that the essential oil from the leaves of *Juniperus oxycedrus* has potential value as a source of new compounds capable of targeting the NF-κB signaling pathway in OA chondrocytes. Upon fractionation, a fraction designated F3 proved to be effective in reducing Il-1-induced NF-κB activation and NO production. Since that fraction contains predominantly a racemic mixture of (+)- and (−)-α-pinene, we compared its ability to inhibit those Il-1-induced responses with those of pure commercial racemic α-pinene, (+)- and (−)-α-pinene enantiomers, as well as with that of turpentine oil, another natural source of racemic α-pinene. β-pinene was also tested.

Methods: The essential oil from *Juniperus oxycedrus* leaves was fractionated by liquid chromatography and analyzed by g*as chromatography* and *gas chromatography-mass spectroscopy*. α-pinene constitutes approximately 92% of F3. Human chondrocytes were isolated from the femoral condyles of multi-organ donors and used to evaluate the ability of F3, turpentine oil and pure pinene isomers to inhibit Il-1-induced NO production. The human chondrocytic cell line, C-28/I2, was used to evaluate NF-κB activation. Both cell culture types were treated for 30 min with the essential oils or fraction, in two different dilutions, followed by treatment with Il-1β (30 ng/ml) for 30 min or 18 h to evaluate, respectively, the cytoplasmic levels of IκB-α by western blot, or NO production by the Griess reaction. To determine whether the test compounds have any direct effect on iNOS enzyme activity, NO production was evaluated in cell cultures pretreated with Il-1, 30 ng/ml, to induce the expression of the enzyme before the addition of the test compounds. The MTT reduction assay was used to rule out cytotoxic effects.

Table 1: Effect of pretreatment with natural and synthetic pinene isomers on Il-1-induced NO production

	NO production induced by Il-1β 30 ng/ml	
	Test compound dilution 1:7500	Test compound dilution 1:5000
No pretreatment	100.0±6.0%	
Commercial racemic α-pinene	58.5±7.5%	40.6±3.4%
(+)-α-pinene	56.2±10.8%	30.1±2.9%
(−)-α-pinene	63.4±7.8%	57.3±6.0%
β-pinene	82.9±13.7%	76.7±14.7%
F3	51.4±7.3%	11.3±0.4%
Turpentine oil	71.8±3.5%	51.2±1.9%

Results: As shown in the table, F3, containing racemic α-pinene from natural origin, is the most effective in reducing Il-1-induced NO production (11.3%±0.4). When added to the chondrocyte cultures 18 h after addition of Il-1, F3 also reduced NO production, although with a lower magnitude than when added to the cell cultures before Il-1. Among the pure compounds, (+)-α-pinene was the most effective in reducing Il-1-induced NO production (30.1%±2.9), either when added before or after Il-1. β-Pinene was only slightly active. F3 and (+)-α-pinene were also the most effective in preventing Il-1-induced IκB-α degradation.

Conclusions: (+)-α-Pinene is more active than its (−) enantiomer. Since F3 achieved a greater inhibition of both NF-κB activation and NO production than the commercial racemic α-pinene or the (+)-α-pinene enantiomer, it is possible that other minor components of that fraction will also be active. The results obtained also indicate that α-pinene acts